



Seasonality in diet and feeding habits of the endemic Chala tilapia (*Oreochromis hunteri*) and two introduced tilapiine cichlids in Lake Chala, East Africa

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Abstract *Oreochromis* cf. *korogwe* and *Coptodon rendalli*, two tilapiine fishes introduced to Lake Chala (Kenya/Tanzania) ~ 40 years ago, may negatively affect the endemic Chala tilapia (*Oreochromis hunteri*) by competition for food. However, the actual threat posed by the introduced cichlids cannot be assessed without data on the local feeding habits of all three species. This paper describes the diet of *O. hunteri* and both introduced species, focusing on seasonal changes in food-source availability.

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Microscopic analysis of gut content in 35 fishes collected over a 20-month period was compared with the composition of littoral food sources and seasonal variation in the limnetic phytoplankton and complemented with exploratory stable-isotope analysis of fish tissue. We found that all three tilapiines in Lake Chala are herbivorous, and during most of the year, they feed on algae and organic detritus on and between rocks in the littoral zone. However, in July–September they migrate to open water to feed on blooming phytoplankton. Interspecific differences in gut content and stable-isotope composition suggest that *O. hunteri* may have a competitive advantage over the two newcomers by making better use of this seasonal extra food resource. However, this advantage may erode when continuing climate change starts affecting the bloom's predictability.

Keywords Fish introduction · Food competition · Gut content · Stable-isotope composition

Introduction

Introductions of non-native species to natural systems are a major concern in conservation biology, as they often cause considerable hazards for the system's indigenous species (Vitousek et al., 1997; Strayer, 2010). A prime example is the extensive transfer of

tilapiine fishes between continents and regions for the purpose of aquaculture (Eknath & Hulata, 2009; FAO, 2014), which has caused widespread decline and even extinction of native ichthyofauna worldwide (Canonico et al., 2005). Tolerant of a wide range of environmental conditions, introduced tilapias often thrive in their new habitat and may then either outcompete or hybridise with the indigenous species (D'Amato et al., 2007; Angienda et al., 2011; Genner et al., 2013; Deines et al., 2014). Among African tilapias, probably the most famous case is the strong decline of the native *Oreochromis esculentus* (M. Graham) and *O. variabilis* (Boulenger) in Lake Victoria after the introduction of *O. niloticus* (Linnaeus) (Ogutu-Ohwayo, 1990; Witte et al., 1991; Goudswaard et al., 2005).

The Chala tilapia *Oreochromis hunteri* (Günther) is endemic to Lake Chala (locally 'Challa', after a nearby village), a crater lake bridging the border of Kenya and Tanzania near Mt. Kilimanjaro (Fig. 1), and is its only known indigenous fish species. Fish fossils in the lake's bottom sediments indicate that *O.*

hunteri has inhabited Lake Chala for at least 25,000 years (Dieleman et al., 2015, 2019b), but genetic data suggest that it may already have colonised the lake much earlier, not long after the crater basin was formed ~ 250,000 years ago (Dieleman et al., 2019a). *Oreochromis hunteri* is listed on the IUCN red list as 'Critically Endangered' (Bayona et al., 2006) because of its exclusive occurrence in this isolated crater lake and scarce information on the population's overall health. Despite being the type species of its genus (Günther, 1889), until recently the ecology of *O. hunteri* had never been studied. Therefore, little information was available on its breeding and feeding behaviour (Trewavas, 1983), except for the observation by Lowe (1955) of juveniles at the shore feeding on algae and detritus.

Sometime in the second half of the 20th century, possibly as late as the early 1980s, three other cichlid species were introduced to Lake Chala (Dadzie et al., 1988; Seegers et al., 2003). These include a haplochromine cichlid belonging to the *Astatotilapia bloyeti* Sauvage complex (Moser et al., 2018; locally these fishes are known as 'Obonio') and two other tilapiines, the redbreast tilapia *Coptodon rendalli* (Boulenger) (locally 'Kijiji') and an unknown *Oreochromis* species (locally 'Bandia'). Following Seegers et al. (2003), we provisionally refer to the latter species as *O. cf. korogwe* (Lowe) (see Dieleman et al., 2015), whereas Moser et al. (2019) label this taxon as *O. sp. 'blue head'*. Mitochondrial DNA sequencing (of ND2, CR and D-loop loci) indicates that this taxon is actually of mixed descent, with a substantial contribution by *O. urolepis* (Norman) (Dieleman et al., 2019a; Moser et al., 2019). All three introduced species have survived until the present and appear to be thriving. Considering that the two introduced tilapias especially have similar ecological requirements to *O. hunteri*, it can be expected that their use of local resources interferes with the native species' use of those same resources. Moreover, the steep-sloping rocky crater walls and deep open water of Lake Chala provide limited opportunity for spatial niche segregation. Morphometric and genetic analyses indicate that no hybridisation has thus far occurred between the introduced and native *Oreochromis* in Lake Chala (Dieleman et al., 2015, 2019a; Moser et al., 2019). Moser et al. (2019) also made a first attempt to characterise the habitat distribution of the three tilapias in Lake Chala, based on experimental fishing

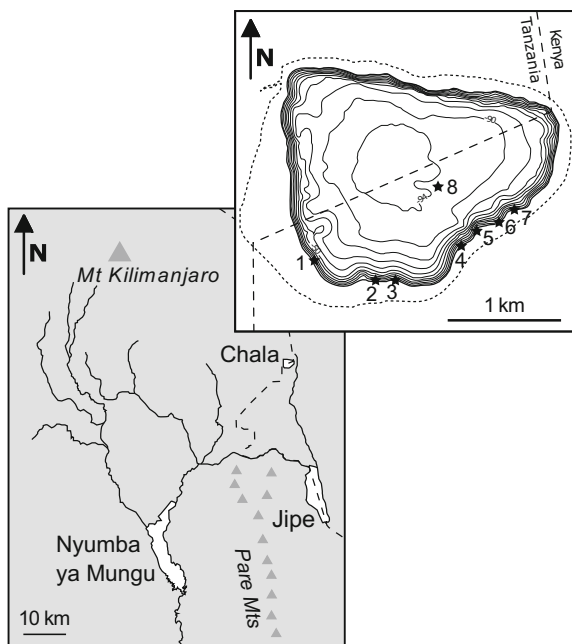


Fig. 1 Map of the upper Pangani River basin in northern Tanzania with location of the region's three major surface waters and bathymetric map of Lake Chala within its crater basin (stippled line) bridging the Kenya-Tanzania border (hatched line). Littoral food-source samples were collected at locations 1 through 7; limnetic phytoplankton samples at location 8

during a single campaign in November 2014. Stable-isotope analysis of specimens collected at that time indicated significant distinction between them in feeding niche.

The versatility of tilapiine cichlids is not only advantageous when coping with new competitors, it is also highly useful in aquatic environments with marked seasonal or longer-term variation in food availability (Lowe-McConnell, 1987). In deep tropical lakes, wind-induced seasonal upwelling can be an important driver of temporary increases in particular food types, and tilapia species have been documented to adapt their feeding behaviour to such events (Spataru, 1976; Man & Hodgkiss, 1977; Spataru & Zorn, 1978). In the case of Lake Chala, the annual phytoplankton bloom which develops during the period of deep water-column mixing between July and September (Barker et al., 2011; Wolff et al., 2011; Buckles et al., 2014; van Bree et al., 2018) creates an opportunity for the Lake Chala cichlids to supplement their diet of near-shore food sources with free-floating algae. We hypothesised that *O. hunteri* may exploit this resource to a different degree than the introduced tilapiines, thus creating a form of niche segregation which may promote their cohabitation.

In the above context, this study documents the diet and feeding habits of *O. hunteri* and the two introduced tilapiines with special emphasis on the influence of seasonal variation in resource availability. For this purpose, data on gut-content composition and associated stable-isotope signatures of fishes collected over a period of 20 months were directly compared with data on the spatial (littoral *versus* limnetic) and seasonal distribution of different food sources. In this way, we aimed to better characterise the precise role of feeding ecology in the present, and potentially the future, impact of the introduced tilapiines on *O. hunteri* in Lake Chala.

Materials and methods

Study area

Lake Chala (03° 19' S, 37° 42' E; Fig. 1) is a deep and permanently stratified crater lake, situated at an elevation of 880 m on the border between Kenya and Tanzania, immediately east of Mt Kilimanjaro. Contained within a steep-sided caldera basin, its rocky

shoreline drops down near-vertically to a depth of *ca* 55–60 m, from where soft fine-grained sediments slope more gently towards a maximum depth of about 90 m in the middle of the lake (Moernaut et al., 2010). For most of the year, oxygenated water extends down to between 20 and 30 m only (Wolff et al., 2011; Buckles et al., 2014), effectively restricting viable fish habitat to the upper half of the rocky crater slopes and the limnetic zone. Due to high water transparency (during most of the year, Secchi-disc values exceed 4 m; van Bree et al., 2018), the submerged rocks receive adequate sunlight and are hence covered with a film of epilithic algae. Crevices between the rocks contain small patches of volcanic sand, which probably increase in areal extent downslope because the sand may completely fill up these crevices at water depths beyond wave-induced turbulence. Trapping the sinking litter of near-shore trees and shrubs, these sandy patches are inhabited by a diverse community of zoobenthos (insect larvae, micro-crustaceans, annelid worms, etc.), at least down to where dissolved oxygen levels allow these invertebrates to live. For most of the year, food for the Lake Chala fishes is limited to this near-shore habitat, since low phytoplankton productivity results in poor feeding conditions throughout the limnetic zone. However, between late June and September the coincidence of strong dry-season monsoon winds with the southern-hemisphere winter minimum in solar insolation (and hence, the lowest lake-surface temperature; van Bree et al., 2018) causes intermittent deep mixing of the water column, down to between 35 and 45 m depth (Wolff et al., 2011; Buckles et al., 2014). These mixing events induce an upwelling of dissolved nutrients that had been accumulating in the hypolimnion, and allow a pronounced bloom of limnetic phytoplankton to develop (Barker et al., 2011; Wolff et al., 2011; van Bree et al., 2018) that may represent a potentially dramatic increase in limnetic feeding opportunity for the local fishes.

Sample collection

Considering the IUCN Red List status of *O. hunteri*, we refrained from prolonged scientific fish collecting in Lake Chala. Instead, during a 20-month period from February 2014 until October 2015, targeted fish samples were collected through purchase from local fishermen who use gill nets suspended from floats, and this on 14 occasions (with each visit lasting 3–4 days)

at approximately monthly intervals. As these fishermen adapt the location and depth of their nets to where large fish are predicted to occur, either near the surface (July–September), > 6 m deep (May–June and November–December) or both, fishing effort can be considered broadly uniform throughout this period. However, fishing in Lake Chala has strongly dwindled over the last decade, mainly due to harassment from alcohol distillers occupying some of the shallow caves inside the crater. All except one of the fishermen currently active in Lake Chala go home to tend their farms during the seasons when large-sized tilapias dwell at depths beyond the reach of their nets.

Our fish collection included 41 full-grown tilapia specimens (> 20 cm standard length) representing all three species: 18 *O. hunteri*, 9 *O. cf. korogwe* and 14 *C. rendalli*. Their guts were dissected immediately in the field to halt digestion, divided into their rostral, middle and caudal parts, and stored in absolute ethanol (99.8%). For stable-isotope analysis, dorsal muscle tissue was collected from 17 specimens using a sterile scalpel and stored frozen until the moment of sample processing. In total, 14 samples of potential littoral food sources were obtained in September 2015 at seven locations on the Kenyan side of the lake (Fig. 1) by scraping off epilithic algal mats from shoreline rocks (12 samples) and by rinsing sand from sandy patches below the water line (two samples). This was done at depths ranging between 0.5 and 2 m, either by reaching out from a boat or by snorkelling. These samples as well were frozen for later analyses. The limnetic phytoplankton was sampled monthly between February 2014 and September 2015, by filling 100 ml bottles with surface water, and at two additional depth intervals (5 and 10 m) between February 2014 and January 2015, using a UWITEC water sampler. Immediately after collection, these water samples were fixed in situ with an alkaline Lugol's solution and formalin.

Laboratory methods

Upon return of all samples to Ghent University, the abundance of food present in the fish guts was first determined qualitatively. Rostral parts filled over their entire length were considered full, whereas guts only partially filled but still providing sufficient material for compositional analysis were treated as half-full. All others (six in total) were considered empty for the

purpose of this study. The middle and caudal parts of all guts only contained strongly digested material and were not analysed. The gut content at the anterior end of each rostral part was transferred to a 2-mL Eppendorf vial and diluted with distilled water. If the anterior end did not contain sufficient material, which sometimes occurred in half-full guts, the first food bolus encountered was used; this was always within the anterior 10 cm of the rostral part. Samples of littoral food sources were similarly diluted in distilled water. One ml of the resulting suspension was pipetted into a sedimentation chamber of 10 ml and left to settle. The samples of limnetic phytoplankton were analysed following the Ütermöhl method (Ütermöhl, 1931, 1958). At least 500 algal/cyanobacterial cells or colonies were counted in each limnetic sample, as recommended by Cocquyt & Vyverman (2005) to adequately capture taxonomic diversity and relative abundance. Counting of gut and littoral food-source samples was limited to 200 cells, colonies or detritus items but, to the extent possible, at the same taxonomic resolution. Identification and counting was done using a Kyowa inverted microscope (gut and littoral samples, at Ghent University) or an Olympus CKX 41 inverted microscope (phytoplankton, at Meise Botanic Garden). All identifications were done at 1000× magnification, using a 100× objective with immersion oil. Algal/cyanobacterial taxa made up the bulk of most studied samples, and were identified to genus level for the dominant taxa and to order level for other taxa. Colonies were counted as one item, but the number of individual cells in each colony was also recorded. For each colony-forming taxon of phytoplankton or epilithic alga, the recorded average number of cells per colony was compared between the guts and the food sources. When this number differed, as was to be expected due to disintegration of colonies inside the gut, the gut colony counts were re-scaled. Macroscopic plant remains were classified as detritus; most of these must originate from terrestrial plant litter because submerged or emergent aquatic macrophytes are almost entirely lacking in Lake Chala. Isolated sponge spicules were occasionally found in the guts, and these were also regarded as detritus, rather than evidence for direct consumption of sponges.

Samples of fish muscle tissue collected for stable-isotope analysis were dried overnight at 60°C and pulverised using a mortar. Subsamples were weighed

to the nearest 0.01 mg before being put into tin capsules (8 × 5 mm). Most samples were measured in duplicate to assess analytical error. Stable-isotope analysis was carried out at the Isotope Bioscience Laboratory (ISOFYS) of Ghent University. The samples were run for nitrogen (N) and carbon (C) isotopic ratios, which are expressed in delta notation:

$$\delta^{15}\text{N} \text{ or } \delta^{13}\text{C} = \left[\left(R_{\text{sample}} / R_{\text{standard}} \right) - 1 \right] \times 1000$$

with $R = {}^{15}\text{N}/{}^{14}\text{N}$ or ${}^{13}\text{C}/{}^{12}\text{C}$ relative to a standard, which is atmospheric nitrogen for N and Pee Dee Belemnite limestone for C.

Data analyses

Numerical data analyses were performed in R version 3.3.2 (R Development Core Team, 2016). Species accumulation curves (package ‘vegan’ v.2.4-3; Oksanen et al., 2017) were used to confirm that counting 200 algal/cyanobacterial cells, colonies or detritus items sufficed to capture the fractional composition of ingested food; and that enough fish specimens were analysed for comprehensive assessment of each of the three fish species’ diet in Lake Chala. To assess seasonal differences in gut content, guts of each of the three cichlid species were considered collectively per season. Guts of fishes caught during the mixing period (July to September) were pooled into one group, and those collected during the predominantly stratified period (October to June) into another group, but separately per year. Our 20-month monitoring of gut content therefore resulted in observations from four consecutive periods (hereafter ‘seasons’) identified as Stratified-14, Mixing-14, Stratified-14/15 and Mixing-15. Average gut contents for these periods were compared with multivariate techniques, using the packages ‘vegan’ and ‘MASS’ v.7.3.45 (Venables & Ripley, 2002). A Bray–Curtis similarity matrix was generated based on square root transformed counts of the gut contents. The homogeneity of spread among samples was checked, as necessary prerequisite for permutational multivariate analysis of variance (PERMANOVA). Seasonal differences in diet were tested using 999 PERMANOVA permutations, followed by pairwise comparison in post hoc tests. The results were visualised using non-metric multidimensional scaling (NMDS).

To relate seasonal differences in diet with seasonal variation in available food sources, average gut-content compositions for each sampled month (here, of the three fish species combined) were compared with the composition of littoral food sources, and of limnetic phytoplankton from the equivalent months. Counts were summed per month and divided by the total number of food items recorded in that month to calculate a percent numerical value (%N; after Hyslop, 1980). The relative abundance of different algal/cyanobacterial taxa in phytoplankton was estimated in similar manner. The samples from 0, 5 and 10 m depth available for the period from February 2014 to January 2015 were pooled into a single %N value per month, whereas water-surface (0 m) samples were used for the period from February 2015 to September 2015. As littoral samples were available only for September 2015, the reported %N for rock scrapes and sandy patches are averages of the 12 rock and two sand sites sampled at that time. The above-mentioned metrics were calculated both at the phylum level (with separate categories for detritus and sand grains) and at the highest taxonomic resolution (i.e. lowest taxonomic level) feasible. The results were visualised using bar plots.

Generalised (i.e. long-term average) diet differences between the three tilapiine species were assessed using $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values. A possible influence of high lipid values on $\delta^{13}\text{C}$ results can be controlled for by simultaneous lipid extraction or mathematical normalisation (Post et al., 2007). Animal lipid content has a strong relationship with the C:N ratio, and a C:N ratio < 3.5 (corresponding to a 5% lipid content) is preferred (Post et al., 2007). None of our fish specimens crossed that threshold, hence normalisation of the $\delta^{13}\text{C}$ values was deemed unnecessary. ANOVA, followed by post hoc Tukey tests, were used to test the significance of differences in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values between the fish species. Diet disparity (i.e. the range of isotope values) was assessed for each species by calculating the mean Euclidean distance of each point in the $\delta^{13}\text{C}/\delta^{15}\text{N}$ plot to its group centroid, and then using the sum of variances on the two axes.

Differences in gut composition between species was compared using PERMANOVA analysis, after checking the assumption of homogeneity and using the same similarity matrix as for seasonal comparisons. Results were again visualised using NMDS. The

dietary importance of individual food items was determined per fish species and per season using %N values, and results were visualised in bar plots to quantitatively assess the differences in diet between species.

Results

Seasonal variation in gut content and food sources

The guts of 15 *O. hunteri*, 8 *O. cf. korogwe* and 12 *Coptodon rendalli* from Lake Chala (35 fishes in total) together contained 28 different prey types. Besides coarse plant detritus and accidentally ingested sand grains, these include two types of Cyanobacteria (blue-green algae) and 24 types of eukaryotic algae belonging to the Bacillariophyta (diatoms), Chlorophyta (green algae), Chrysophyta, Cryptophyta, Dinophyta and Euglenophyta (Supplementary Table S1). Algae and Cyanobacteria amounted to between 45% and 95% of the analysed gut contents. The 29 analysed samples of limnetic phytoplankton together contained 27 different potential prey types, namely three types of Cyanobacteria and 24 types of eukaryotic algae (Supplementary Table S2). The 12 littoral rock-scrape samples and two littoral sand-patch samples together contained, respectively, 24 and 18 different potential prey types (Supplementary Table S3).

A two-dimensional NMDS of gut-content data (Fig. 2) generated a sample distribution with acceptable stress values (0.16), and can hence be considered to adequately represent the observed variation in gut-content composition (Table 1). This shows that gut content varied greatly between successive seasons, whereas interspecific differences between the three tilapia species were less distinct. Sample homogeneity differed significantly between seasons ($F = 6.36$, $P < 0.01$), but post hoc Tukey tests revealed only one significant pairwise distinction, namely between the seasons Stratified-14/15 and Mixing-15 (Table 2). PERMANOVA assumptions are therefore met for the majority of inter-season comparisons, albeit that the results of this one comparison should be treated with caution. Overall we found a highly significant impact of seasonality on diet composition ($F = 4.63$, $P < 0.01$), and post hoc testing revealed that all pairwise differences between samples from fishes collected during the main mixing periods (Mixing-14

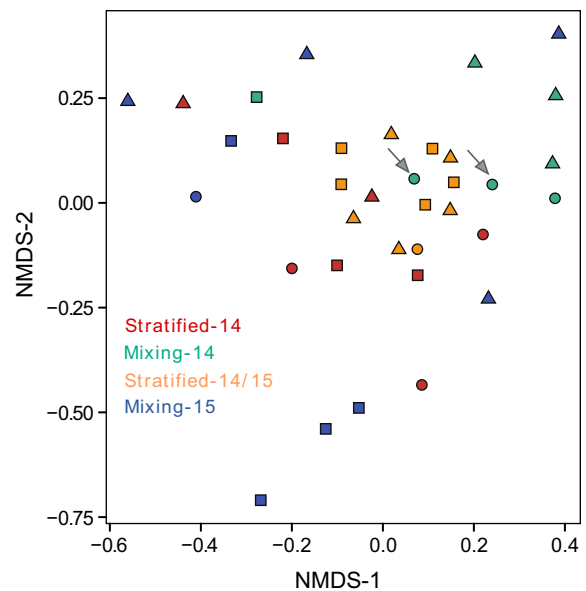


Fig. 2 Non-metric multidimensional scaling ordination (NMDS) visualising the variation in gut content of full-grown specimens from the three tilapia species inhabiting Lake Chala (symbols) during stratified and mixing periods in 2014–2015 (colours): *Oreochromis hunteri* (triangles), *O. cf. korogwe* (circles) and *Coptodon rendalli* (squares). Arrows point to *O. cf. korogwe* specimens collected during the mixing season of 2014, with gut compositions not unlike those of specimens collected during the stratified seasons

and Mixing-15) and those from predominantly stratified periods (Stratified-14 and Stratified-14/15) were borderline to highly significant at the Bonferroni-corrected 5% confidence interval (Table 2).

To better comprehend the sources of seasonal variation in gut content, the relative abundances of different food types in the fishes' guts were compared with those of the limnetic and littoral food sources, first at the phylum level (Fig. 3) and then at higher taxonomic resolution (Fig. 4). A clear pattern is that the proportion of detritus and sand grains present in the guts (Fig. 3b) was much lower during mixing-season months (July–September; 6–13%) than during the rest of the year (27–53%). All phyla of algae/cyanobacteria, except the Chrysophyta, were found to occur in both the limnetic and littoral food sources. In contrast, the detritus and sand in the fish guts have an exclusively littoral origin, since these foods represent almost half of the littoral food sources (Fig. 3c), but are predictably absent in the open surface water. The abundant Chlorophyta in gut contents from during the 2014 mixing season (30–50%; Fig. 3b) are mostly

Table 1 Overview of fish specimens collected in Lake Chala at monthly intervals between February 2014 and September 2015. Values indicate the number of specimens available for gut analysis per month and per species, and the textstyle reflects the relative abundance of gut content: full (**bold type**), half-full (regular type), or empty (*italics*). Dash symbols indicate that no specimens of that species could be collected

Month	<i>Oreochromis hunteri</i>	<i>Oreochromis cf. korogwe</i>	<i>Coptodon rendalli</i>	Total #
February 2014	2	<i>1</i>	1	4
March 2014	1	1	1	3
April 2014	<i>1</i>	1	<i>1</i>	3
May 2014	<i>1</i>	1	1	3
June 2014	–	–	–	–
July 2014	1	1	–	2
August 2014	1	1	–	2
September 2014	1	1	1	3
October 2014	–	–	–	–
November 2014	–	–	–	–
December 2014	1	1	1	3
January 2015	1	–	<i>1</i>	2
February 2015	1	–	1	2
March 2015	–	–	–	–
April 2015	1	–	1	2
May 2015	–	–	–	–
June 2015	1	–	1	2
July 2015	–	–	–	–
August 2015	<i>1</i>	–	–	1
September 2015	4	1	4	9
# Specimens	18	9	14	41
# Analysed	15	8	12	35
# Data months	12	8	9	13

Table 2 Bonferroni-adjusted *P* values of post hoc homogeneity Tukey tests on differences in beta-diversity of gut-content composition grouped per season (above diagonal) and of post hoc PERMANOVA pairwise comparisons of Bray–Curtis dissimilarity matrixes based on gut-content composition

grouped per season (below diagonal), and this for four seasons: February–June 2014 (Stratified-14), July–September 2014 (Mixing-14), October 2014–June 2015 (Stratified-14/15) and July–September 2015 (Mixing-15)

	#	Stratified-14	Mixing-14	Stratified-14/15	Mixing-15
Stratified-14	9		0.91	0.16	0.24
Mixing-14	7	0.01*		0.54	0.07
Stratified-14/15	10	0.47	< 0.01*		< 0.01*
Mixing-15	9	0.09	0.02*	< 0.01*	

Asterisks (*) indicate statistical significance at the adjusted 5% level

represented by *Tetraedron* Kützing (Fig. 4b), consistent with high *Tetraedron* abundance in the limnetic phytoplankton at that time (Fig. 4a). On the other hand, the chlorophyte *Treubaria* C. Bernard is

common in the limnetic phytoplankton from July 2014, but rare in the guts of fish collected at that time. The proportion of Bacillariophyta (diatoms) in the guts throughout the monitoring period is variable

Fig. 3 Percent abundance of algal and cyanobacterial taxa (identified at the phylum level), terrestrial plant detritus, and sand in **a** limnetic phytoplankton per sampled month, **b** full-grown tilapiine fish guts per sampled month and **c** littoral food sources. The latter include epilithic algae on rock faces (rock) and sand patches in between the rocks (sand). We refer to Tables S1–S3 for the exact values represented here as colour hues

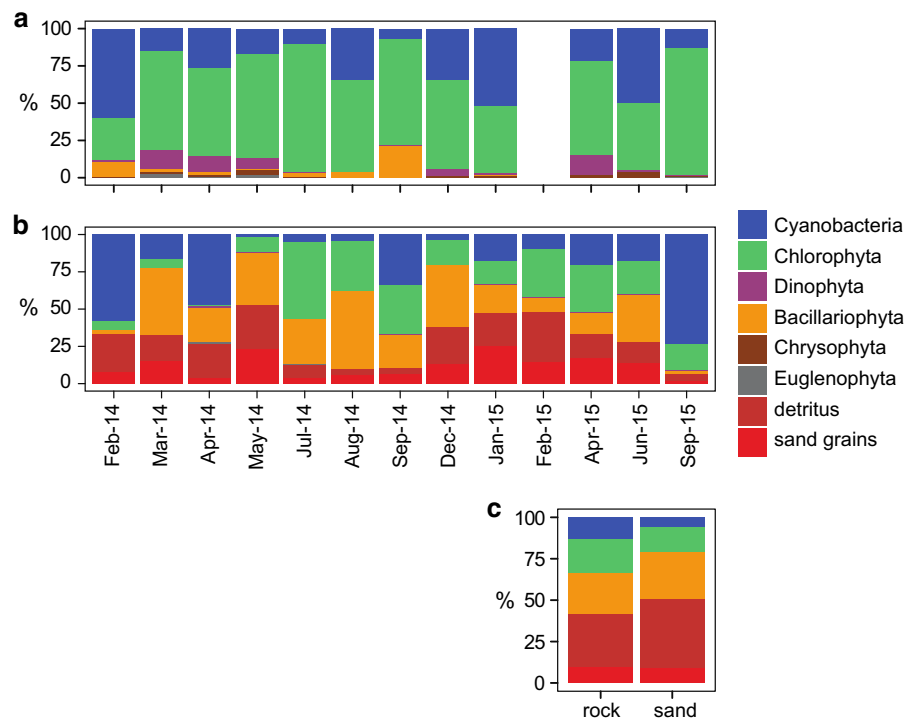
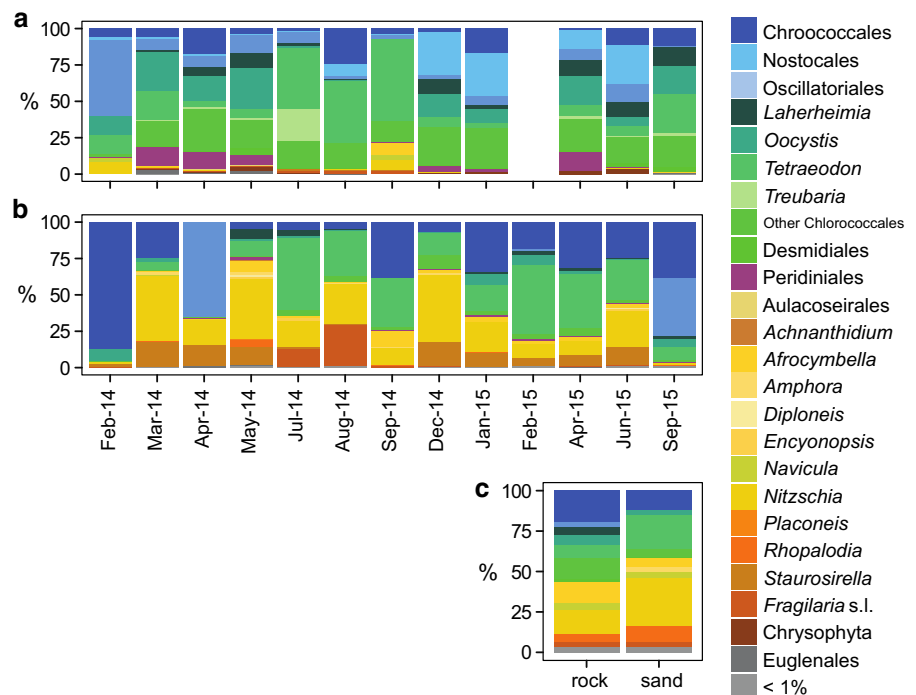


Fig. 4 Percent abundance of algal and cyanobacterial taxa identified at the highest feasible resolution (genus or order) in **a** limnetic phytoplankton per sampled month, **b** full-grown tilapiine fish guts per sampled month and **c** littoral food sources, as in Fig. 3. We refer to Tables S1–S3 for the exact values represented here as colour hues



(2.4–52.5%; Fig. 3b), but even more so is their composition at the genus level (Fig. 4b). A significant part of gut contents during the predominantly stratified

season consists of *Nitzschia* Hassall (10–45%) and *Staurosirella* D. M. Williams (5–18%). In the case of *Nitzschia*, this can be explained by its prominence in

the littoral food sources (15–30%). The need of *Staurisirella* for a firm substrate argues for a predominantly littoral distribution, yet it represents only a small fraction of the littoral samples ($\sim 0.5\%$). Notably, *Staurisirella* also occurs in the limnetic zone during the mixing season (Fig. 4a), when zooplankton faecal pellets provide abundant substrate for *Staurisirella*. Its abundance in fish guts from during the mixing season is nevertheless modest (0–1%), in favour of *Fragilaria* in July–August (12–28%) and *Afrocybella* Krammer (more specifically the endemic species *A. barkeri* Cocquyt & Ryken (2016)) in September (11%), two diatom taxa which also peak in the limnetic phytoplankton at that time (Fig. 4a).

Trends in gut-content composition during the mixing season of 2015 were different from those of 2014. With the caveat that only fishes caught in September were available, their guts ($n = 9$) contained mostly Cyanobacteria (Figs. 3 and 4), except one *O. hunteri* specimen of which the gut was filled with Chlorophyta. All fishes collected at that time have at least some gut content, but most often the food boluses were interspersed with large empty stretches. Absolute abundances of the major groups of algae/cyanobacteria in the phytoplankton sampled at monthly intervals (Fig. 5) show that the ‘customary’ dry-season diatom bloom in Lake Chala (Wolff et al., 2011) failed to develop in 2015. Also, some other important

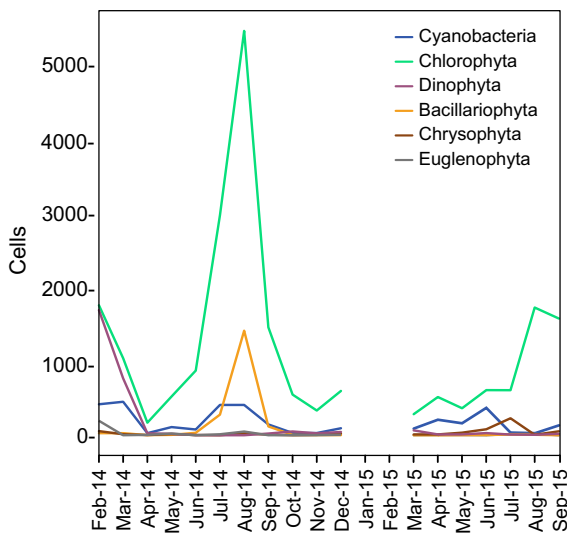


Fig. 5 Total abundance (cell counts in 100 ml surface water) of the six most common algal/bacterial phyla in limnetic phytoplankton from Lake Chala, sampled monthly between February 2014 and September 2015 (except January–February 2015)

compositional shifts in the limnetic phytoplankton are not reflected well in fish-gut contents, such as the high abundances of Dinophyta and the chlorophyte *Oocystis* Nägeli ex A. Braun from March to May 2015, and elevated abundances of *Nostocales* (Cyanobacteria; here mainly *Cylindrospermopsis* Seenayya & N. Subba Raju) in December 2014 and January 2015 (Figs. 3 and 4). Overall, however, fish-gut compositions from during the mixing season resemble the limnetic phytoplankton at that time, whereas guts from fishes collected at other times of the year contain a higher proportion of littoral food sources.

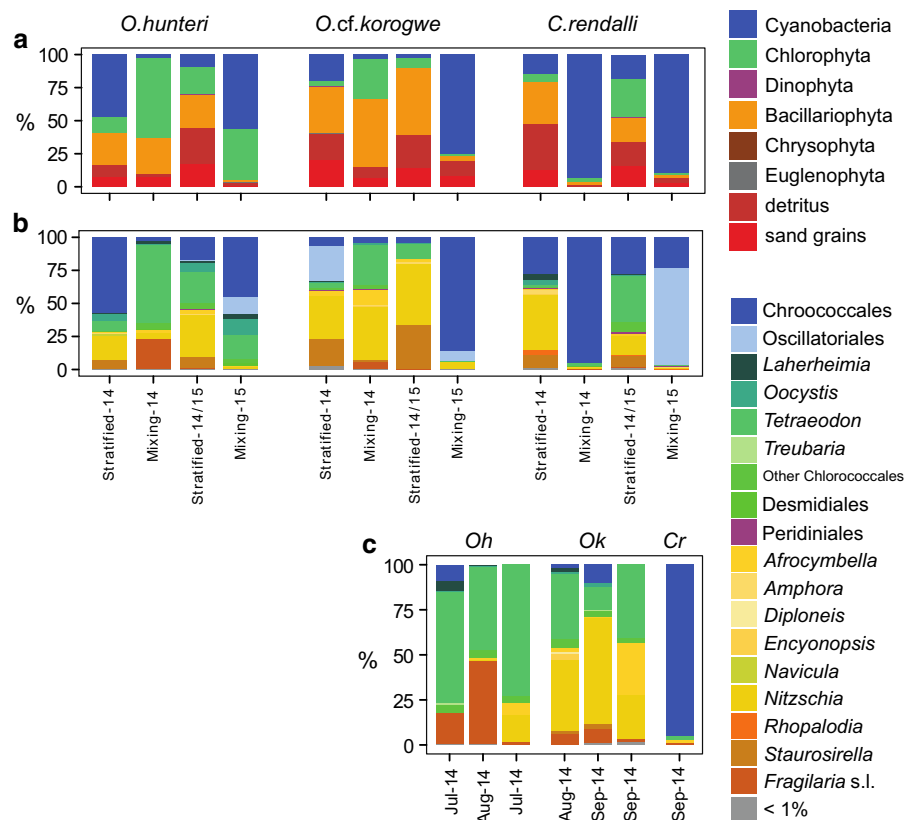
Interspecific differences

When grouped per fish species, no significant difference in the homogeneity of gut contents was found ($F = 0.31$, $P = 0.73$), fulfilling assumptions for PERMANOVA analyses. PERMANOVA indicated a borderline significant difference in gut-content composition between the three species ($F = 1.90$, $P = 0.05$), but in post hoc tests none of the pairwise differences were found to be significant at the Bonferroni-corrected 5% level (Table 3). The sample sizes available for this study did not allow us to simultaneously test for the influences of species and season. However, cumulative bar plots taking into account both factors (Fig. 6) nevertheless reveal interesting interspecific differences. Although all species display the seasonal shift between littoral and limnetic food sources (Fig. 6a), during the mixing season of 2014, the diet of *O. hunteri* contains a much higher contribution of the algae *Tetraedron* and *Fragilaria* Lyngbye s.l. (Fig. 6b) which are blooming at that time (Fig. 4a). In contrast, *O. cf. korogwe* specimens caught during that time (particularly those from July and August 2014, $n = 2$; Fig. 6c) show a diet composition similar to that observed in all species during the predominantly stratified season. This is also reflected in the NMDS plot (Fig. 2), where these two *O. cf. korogwe* specimens cluster with specimens collected during the stratified season. Only the *O. cf. korogwe* individual caught in September 2014 contains higher abundances of the limnetic algae blooming at that time, such as *Afrocybella* and *Tetraedron*. In contrast, the gut content of the single *C. rendalli* specimen available from during the 2014 mixing season consisted mostly of cyanobacteria. Also some interspecific differences can be observed during the

Table 3 Bonferroni-adjusted P-values of post hoc PERMANOVA pairwise comparisons of Bray–Curtis dissimilarity matrices based on gut-content composition, grouped across all samples analysed per species as indicated in Table 1

	<i>Oreochromis hunteri</i>	<i>Oreochromis cf. korogwe</i>
<i>Oreochromis hunteri</i>	–	0.19
<i>Coptodon rendalli</i>	0.30	0.76

Fig. 6 **a** Percent abundance of algal and cyanobacterial phyla, plant detritus and sand in guts of each of the three Lake Chala tilapiines separately, grouped per season as defined in Fig. 2. **b** Percent abundance of algal/cyanobacterial genera and orders in these guts. **c** Percent abundance of algal/cyanobacterial genera and orders in these guts, for each month of the 2014 mixing season separately. *Oh* *Oreochromis hunteri*, *Ok* *O. cf. korogwe*, *Cr* *Coptodon rendalli*. We refer to Tables S4–S5 for the exact values represented here as colour hues



stratified season, such as a higher abundance of the diatom *Staurosirella* in guts of *O. cf. korogwe* than in the other two tilapias.

The total range of $\delta^{13}\text{C}$ values measured from muscle tissue of all sampled Lake Chala tilapias (6.18; $n = 17$) is wider than for $\delta^{15}\text{N}$ (2.88; Fig. 7). In addition, the three tilapiine species differed significantly in average values for both $\delta^{15}\text{N}$ ($F = 19.12$, $P < 0.01$) and $\delta^{13}\text{C}$ ($F = 4.50$, $P = 0.03$). Average $\delta^{15}\text{N}$ values are higher for *O. hunteri* (10.11) and *C. rendalli* (10.22) than for *O. cf. korogwe* (8.02), whereas $\delta^{13}\text{C}$ values for *O. hunteri* (-24.60) were on average lower than for both *O. cf. korogwe* (-21.84) and *C. rendalli* (-22.76).

Discussion

Our collection of Lake Chala tilapiine fishes represents 14 of the 20 months of lake monitoring between February 2014 and September 2015 (Table 1). Of the 6 months when no fishes were available for purchase at the time of our visits, five are during the stratified season (Table 1) when the fish are believed to reside in deep water very close to shore and are hence more difficult to catch. The sixth occurred in July 2015, when the mixing season phytoplankton bloom developed only in August and contained few diatoms (Fig. 5). Further, all sample sizes are relatively modest: on average 2.8 fishes (range 1–9) were

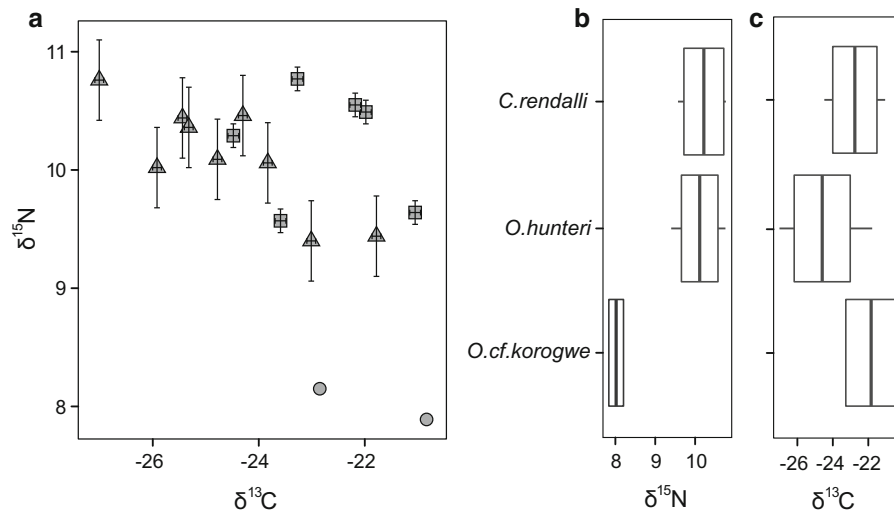


Fig. 7 a Carbon and nitrogen stable-isotope bi-plot for muscle tissue of full-grown *Oreochromis hunteri* (triangles; $n = 9$), *O. cf. korogwe* (circles; $n = 2$) and *C. rendalli* (squares; $n = 6$) in Lake Chala, with error bars representing the standard deviation

obtained per month, and separately the three species are represented in 12 (*O. hunteri*), 8 (*O. cf. korogwe*) and 9 (*C. rendalli*) out of the 20 months. Consequently, certain potentially important factors such as ontogenetic diet shifts (Ribbink, 1990; Piet, 1998; Njiru et al., 2004) could not be properly assessed. We therefore stress that while our study provides first insights into temporal variation in the diet and feeding habits of the endemic *O. hunteri* (as well as the two introduced tilapias) in Lake Chala, our inferences from the available data remain prudent.

A first pertinent result is that the 31 fish guts analysed contained no evidence for live consumption of (macroscopic) animals, not from the rocks nor macro- or meiobenthos from the sand patches nor pelagic zooplankton. If the examined fishes had recently consumed crustacean zooplankton (copepods, water fleas), we certainly would have recovered some diagnostic fragments of their chitin exoskeletons. The same is true for the aquatic insect larvae which the fish might have plucked from the rock face or ingested together with organic bottom debris. The ingested remains of fully soft-bodied benthic organisms, such as nematodes and annelid worms, are likely to degrade more rapidly than those of insects or crustaceans. However, they would not degrade more rapidly than many of the soft-celled algae and cyanobacteria that we recovered sufficiently intact

on data from two replicate samples. **b** and **c** Box plots of the mean, standard deviation and total range (min–max) values of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ for each species

for identification at the genus level. Thus, our gut-content data strongly indicate that consumption of animals by any of the three tilapiines in Lake Chala is accidental only, and that they can be considered negligible as food items in their diet. Like most other tilapias (Trewavas, 1983), the three species currently inhabiting Lake Chala have an (almost) exclusively herbivorous diet, supplemented with plant detritus throughout the year.

Secondly, the diet of all three tilapiines displays major temporal variability that is much more significant than any interspecific differences between them. Strongly linked to the seasonal fluctuation in phytoplankton abundance, this temporal variability in the fishes' diet highlights the important role that temporary resource availability can play in foraging behaviour. At times when the water column is stratified (here, February–June 2014 and October 2014 until June 2015), all three tilapias appear to depend exclusively on littoral food sources. During the mixing season (July–September in both 2014 and 2015), the limnetic phytoplankton bloom of green algae (Chlorophyta) and diatoms (Bacillariophyta) provides sufficient extra food to temporarily draw fishes towards the open water (Figs. 3 and 4). Notably, the considerable limnetic Dinophyta bloom which occurred in February–March 2014 (but may have started before that time; Fig. 5) was not exploited,

indicating that Lake Chala tilapias may prefer even scarce littoral food sources over venturing out to ingest abundant Dinophyta. Alternatively, their migration to the open water may be triggered by an external cue, such as the deepest annual injection of oxygenated water at the end of June (Wolff et al., 2011; van Bree et al., 2018).

Also notable is the scarcity of the chlorophyte *Treubaria* in guts of fish collected in July 2014, despite its high abundance in the water column at that time (Fig. 4). Other planktivorous *Oreochromis* species, such as *O. esculentus*, are known to mechanically process large phytoplankton items after ingestion, using their pharyngeal jaws (Greenwood, 1965; Trewavas, 1983). It thus appears feasible that the large *Treubaria* cells ($\sim 40 \mu\text{m}$, including spines) are broken beyond recognition before entering the gut. This contrasts with the silicified diatom frustules, which may pass the pharyngeal jaws relatively intact.

Marked differences in gut-content composition were found between the mixing seasons of 2014 and 2015. The relatively modest phytoplankton bloom in 2015 (Fig. 5) is reflected in low food abundance in all but one of the 10 examined guts (Table 1), indicating modest consumption in the days before capture. Although guts from September 2015 contain some littoral material (detritus, sand grains; Fig. 3b) it is much less common than during stratified season months, suggesting that the fish were indeed externally triggered to venture out into open water but found sub-optimal feeding conditions there.

Importantly, all three tilapiines display the same seasonal shift in feeding habit (Fig. 6a), indicating that the introduced species have learned to exploit the seasonality in food sources characteristic for Lake Chala, and consequently have a diet now strongly overlapping with that of *O. hunteri* (cf. Moser et al., 2019). Although by itself not sufficient to demonstrate resource competition (Zengeya et al., 2011), this observation provides strong circumstantial evidence that today's population of *O. hunteri* is experiencing serious competition for food, particularly because of the very modest productivity of Lake Chala (as inferred from its transparency being exceptionally high for an African crater lake; Nankabirwa et al., 2019). Indeed, averaged over the year, no significant interspecific differences in gut-content composition could be found. Nevertheless, the stable-isotopic signature of *O. hunteri* is distinct with lower $\delta^{13}\text{C}$

and higher $\delta^{15}\text{N}$ than the non-native species, suggesting at least some form of resource partitioning between them (Zengeya et al., 2011). At face value, and consistent with the results of Moser et al. (2019), our isotopic data (Fig. 7) indicate that *O. hunteri* feeds on isotopically more depleted carbon sources than both introduced tilapias, and at a higher mean trophic level, at least compared to *O. cf. korogwe*. Phytoplankton is known to have on average lower $\delta^{13}\text{C}$ values than epilithic algae and terrestrial plant detritus (Finlay & Kendall, 2007). Specifically for Lake Chala, Hurrell et al. (2011) reported very low $\delta^{13}\text{C}$ values (-28 to -37‰) for the diatom genera *Nitzschia* and *Afrocybella*, which dominate the phytoplankton bloom. So it appears fair to conclude that the relatively low $\delta^{13}\text{C}$ values as well as greater isotopic disparity among *O. hunteri* individuals (Fig. 7) may be explained by their more targeted, but potentially variable, seasonal feeding on the limnetic phytoplankton. The suggestion, based on slightly elevated $\delta^{15}\text{N}$ values, that *O. hunteri* (as well as *C. rendalli*) feeds at a higher mean trophic level than *O. cf. korogwe* (Moser et al., 2019) appears to conflict with our gut-content data showing a complete lack of macroscopic animal foods. Considering also the overall low abundance of crustacean zooplankton in Lake Chala (mainly copepods; Verschuren et al., unpublished data), the elevated $\delta^{15}\text{N}$ values of *O. hunteri* may well reflect its accidental ingestion of planktonic protozoans, which can represent a sizable component of the limnetic food web in African lakes (Ngochera & Bootsma, 2011), but of which the abundance and distribution in Lake Chala is as yet undocumented. If true, then also the elevated $\delta^{15}\text{N}$ values of *O. hunteri* may reflect its feeding being spatially segregated from that of *O. cf. korogwe* during part of the year. In any event, the average $\delta^{15}\text{N}$ value of *O. hunteri* in this study (10.11 ; $n = 9$) and in the study by Moser et al. (2019) (8.0 ; $n = 23$) remains well below values typically recorded for truly omnivorous fishes in other African lakes (Ngochera & Bootsma, 2011).

Looking in detail at the seasonal sequence of ingested food, differences between species are most prominent in guts from the mixing season (Fig. 6c), potentially indicating a spatial segregation in feeding during the phytoplankton bloom only. Indeed, during the mixing season of 2014, *O. hunteri* guts contained the predominantly limnetic chlorophyte *Tetraedron* from July onwards, gaining the diatoms *Nitzschia* and

Afrocybella only when these taxa became common in the open water phytoplankton (compare Fig. 6c with Figs. 4a and 5). In contrast, *O. cf. korogwe* guts contained less *Tetraedron* throughout this period, indicating that it either lingered in the littoral or migrated to the open water only when *Afrocybella* peaked there in September (compare Figs. 6c with Fig. 4a). The guts of all five *C. rendalli* specimens collected during the mixing period (one in September 2014 and four in September 2015) contained almost nothing but cyanobacteria (either Chroococcales or a mix of Chroococcales and Oscillatoriales, besides small amounts of plant detritus), implying that this species does venture out to the open water but apparently does not feed on the green algae or diatoms.

Taken together, our gut-content and stable-isotope data confirm the finding by Moser et al. (2019) of major overlap in habitat distribution and stable-isotope signatures, and hence food resource use, between the three tilapiines living together in Lake Chala. In addition, we can conclude from our 20-month gut-content time series that *O. hunteri* currently appears to be taking greater advantage of the food resource provided by the annual phytoplankton bloom than the introduced tilapiines. When abundant, this easily ingestible food is likely preferable over perennially modest littoral food sources that require active search, and involve greater inference competition with other fishes. Although the two introduced species also exploit the full range of available food sources, they have not (yet) synchronised their feeding behaviour equally well to the strongly seasonal pattern in food availability as has the indigenous *O. hunteri*. If so, this conceivably confers a competitive advantage to *O. hunteri* that may help it to maintain a viable population in Lake Chala despite its forced cohabitation with two tilapias strongly overlapping in ecological requirements. Nevertheless, this advantage may depend on the predictable annual recurrence of a strong bloom of limnetic phytoplankton composed of diatoms and Chlorophyta. Current evidence from (discontinuous) lake monitoring since 2003 (Verschuren et al., unpublished data) indicates that the occurrence of such a bloom is the normal pattern, but not guaranteed. Using sedimentary data, Wolff et al. (2011, 2014) found that the magnitude of mixing season diatom production in Lake Chala is related to and inter-annual climate variability, with wetter and less windy weather conditions during El Niño years

(Black et al., 2003) limiting the amount of deep water nutrients being upwelled during the mixing season and, in turn, curtailing the annual diatom bloom. In this context, the clear impact of the ‘failed’ phytoplankton bloom of 2015 (i.e. no diatoms and 70% less chlorophyta than in 2014) on both the quantity and composition of fish-gut content raises concern that the higher rainfall amounts and variability projected for a warmer late-twenty-first-century East Africa (Held & Soden, 2006; IPCC, 2014) may adversely affect the occurrence of Lake Chala’s annual phytoplankton bloom, and potentially have a greater long-term negative impact on *O. hunteri* than on the introduced tilapiines. *Oreochromis hunteri* has already survived multiple millennia of major climate-driven habitat alteration (Dieleman et al., 2019b), but in all likelihood it has never had to cope with these changes while competing with other fish species for scarce food resources (Dieleman et al., 2015, 2019a). Therefore, anthropogenic climate change, rather than the fish introductions themselves or fishing pressure, may eventually determine the long-term fate of Lake Chala’s endemic tilapia.

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Author contributions JD participated in conceiving the study and the sampling design, gathered and analysed gut contents and littoral food-source samples, interpreted all data and wrote the draft paper. CC gathered and analysed limnetic phytoplankton samples and helped with interpreting those results. WDN participated in study design, provided institutional support and provided comment on the manuscript. DV participated in conceiving the study and interpreting data, and helped optimise the manuscript.

References

- Angienda, P. O., H. J. Lee, K. R. Elmer, R. Abila, E. N. Waindi & A. Meyer, 2011. Genetic structure and gene flow in an endangered native tilapia fish (*Oreochromis esculentus*) compared to invasive Nile tilapia (*Oreochromis niloticus*) in Yala Swamp, East Africa. *Conservation Genetics* 12: 243–255.
- Barker, P., E. R. Hurrell, M. J. Leng, C. Wolff, C. Cocquyt, H. J. Sloane & D. Verschuren, 2011. Seasonality in equatorial climate over the past 25 ky revealed by oxygen isotope records from Kilimanjaro. *Geology* 39: 1111–1114.
- Bayona, J. D. R., E.A. Odhiambo & M. Hanssens, 2006. *Oreochromis hunteri*. In *IUCN Red List of Threatened Species* Version 2006. Available at doi.org/<https://doi.org/10.2305/iucn.uk.2006.rlts.t60634a12389528.en> (accessed 11 April 2018).
- Black, E., J. Slingo & K. R. Sperber, 2003. An observational study of the relationship between excessively strong short rains in coastal East Africa and Indian Ocean SST. *Monthly Weather Review* 131: 74.
- Buckles, L. K., J. W. H. Weijers, D. Verschuren & J. S. Sinninghe Damsté, 2014. Sources of core and intact branched tetraether membrane lipids in the lacustrine environment: anatomy of Lake Challa and its catchment, equatorial East Africa. *Geochimica et Cosmochimica Acta* 140: 106–126.
- Canonico, G. C., A. Arthington, J. K. McCrary & M. L. Thieme, 2005. The effects of introduced tilapias on native biodiversity. *Aquatic Conservation* 15: 463–483.
- Cocquyt, C. & E. Ryken, 2016. *Afrocymbella barkeri* sp. nov. (Bacillariophyta), a common phytoplankton component of Lake Challa, a deep crater lake in East Africa. *European Journal of Phycology* 51: 217–225.
- Cocquyt, C. & W. Vyverman, 2005. Phytoplankton in Lake Tanganyika: a comparison of community composition and biomass off Kigoma with previous studies 27 years ago. *Journal of Great Lakes Research* 31: 535–546.
- D'Amato, M. E., M. M. Esterhuysen, B. C. W. Van Der Waal, D. Brink & F. A. M. Volckaert, 2007. Hybridization and phylogeography of the Mozambique tilapia *Oreochromis mossambicus* in southern Africa evidenced by mitochondrial and microsatellite DNA genotyping. *Conservation Genetics* 8: 475–488.
- Dadzie, S., R. D. Haller & E. Trewavas, 1988. A note on the fishes of Lake Jipe and Lake Chale in the Kenya-Tanzania border. *Journal of East African Natural History and National Museums of Kenya* 192: 46–51.
- Deines, A. M., I. Bbole, C. Katongo, J. L. Feder & D. M. Lodge, 2014. Hybridisation between native *Oreochromis* species and introduced Nile tilapia *O. niloticus* in the Kafue River, Zambia. *African Journal of Aquatic Science* 39: 23–34.
- Dieleman, J., B. Van Bocxlaer, C. Manntschke, D. W. Nyingi, D. Adriaens & D. Verschuren, 2015. Tracing functional adaptation in African cichlid fishes through morphometric analysis of fossil teeth: exploring the methods. *Hydrobiologia* 755: 73–88.
- Dieleman, J., M. Muschick, D. W. Nyingi & D. Verschuren, 2019a. Species integrity and origin of *Oreochromis hunteri* (Pisces: Cichlidae), endemic to crater lake Chala (Kenya-Tanzania). *Hydrobiologia* 832: 269–282.
- Dieleman, J., B. Van Bocxlaer, W. D. Nyingi, A. Lyaruu & D. Verschuren, 2019b. Recurrent changes in cichlid dentition linked to climate-driven lake-level fluctuations. *Ecosphere* 10: e02664.
- Eknath, A. E. & G. Hulata, 2009. Use and exchange of genetic resources of Nile tilapia (*Oreochromis niloticus*). *Reviews in Aquaculture* 1: 197–213.
- FAO, 2014. The state of the world fisheries and aquaculture. Available at <http://www.fao.org/3/a-i3720e.pdf>.
- Finlay, J. C. & C. Kendall, 2007. Stable isotope tracing of temporal and spatial variability in organic matter sources to freshwater ecosystems. In Michener, R. L. K. (ed.), *Stable Isotopes in Ecology and Environmental Science*. Blackwell, Oxford: 283–333.
- Genner, M. J., E. Connell, A. Shechonge, A. Smith, J. Swannstrom, S. Mzighani, A. Mwijage, B. P. Ngatunga & G. F. Turner, 2013. Nile tilapia invades the Lake Malawi catchment. *African Journal of Aquatic Science* 38: 85–90.
- Goudswaard, P. C., F. Witte & E. F. B. Katunzi, 2005. The tilapiine fish stock of Lake Victoria before and after the Nile perch upsurge. *Journal of Fish Biology* 60: 838–856.
- Greenwood, P. H., 1965. Environmental effects on the pharyngeal mill of a cichlid fish, *Astatoreochromis alluaudi*, and their taxonomic implications. *Proceedings of the Linnean Society of London* 176: 1–10.
- Günther, A., 1889. On some fishes from the Kilima-Njaro district. In *Proceedings of the Scientific Meetings of the Zoological Society of London*: 70–72.
- Held, I. M. & B. J. Soden, 2006. Robust responses of the hydrological cycle to global warming. *Journal of Climate* 19: 5686–5699.
- Hurrell, E. R., P. A. Barker, M. J. Leng, C. H. Vane, P. Wynn, C. P. Kendrick, D. Verschuren & F. Alayne Street-Perrott, 2011. Developing a methodology for carbon isotope analysis of lacustrine diatoms. *Rapid communications in Mass Spectrometry* 25: 1567–1574.
- Hyslop, E. J., 1980. Stomach contents analysis: a review of methods and their application. *Journal of Fish Biology* 17: 411–429.
- Lowe-McConnell, R. H., 1987. *Ecological Studies in Tropical Fish Communities*. Cambridge University Press, Cambridge.
- IPCC, 2014. *Climate Change 2014: Synthesis Report*. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change (Core Writing Team, R.K. Pachauri and L.A. Meyer, eds). IPCC, Geneva 151 pp.
- Lowe, R. H., 1955. New species of tilapia (Pisces, Cichlidae) from Lake Jipe and the Pangani River, East Africa. *Bulletin of the British Museum (Natural History) Zoology* 2: 349–368.
- Man, H. S. H. & I. J. Hodgkiss, 1977. Studies on the ichthyofauna in Plover Cove Reservoir, Hong Kong: I. Sequence of fish population changes. *Journal of Fish Biology* 10: 493–503.
- Moernaut, J., D. Verschuren, F. Charlet, I. Kristen, M. Fagot & M. De Batist, 2010. The seismic-stratigraphic record of lake-level fluctuations in Lake Challa: hydrological

- stability and change in equatorial East Africa over the last 140 kyr. *Earth and Planetary Science Letters* 209: 214–223.
- Moser, F. N., J. C. van Rijssel, S. Mwaiko, J. I. Meier, B. Ngatunga & O. Seehausen, 2018. The onset of ecological diversification 50 years after colonization of a crater lake by haplochromine cichlid fishes. *Proceedings of the Royal Society B* 28: 20180171.
- Moser, F. N., J. C. van Rijssel, B. Ngatunga, S. Mwaiko & O. Seehausen, 2019. The origin and future of an endangered crater lake endemic; phylogeography and ecology of *Oreochromis hunteri* and its invasive relatives. *Hydrobiologia* 832: 283–296.
- Nankabirwa, A., W. De Crop, T. Van der Meeren, C. Cocquyt, P.-D. Plisnier, J. Balirwa & D. Verschuren, 2019. Phytoplankton communities in the crater lakes of western Uganda, and their indicator species in relation to lake trophic status. *Ecological Indicators* 107: 105563.
- Ngochera, M. J. & H. A. Bootsma, 2011. Temporal trends of phytoplankton and zooplankton stable isotope composition in tropical Lake Malawi. *Journal of Great Lakes Research* 37: 45–53.
- Njiru, M., J. B. Okeyo-Owuor, M. Muchiri & I. G. Cowx, 2004. Shifts in the food of Nile tilapia, *Oreochromis niloticus*, in Lake Victoria, Kenya. *African Journal of Ecology* 42: 163–170.
- Ogutu-Ohwayo, R., 1990. The decline of the native fishes of Lakes Victoria and Kyoga (East Africa), and the impact of introduced species, especially the Nile perch, *Lates niloticus*, and the Nile tilapia, *Oreochromis niloticus*. *Environmental Biology of Fishes* 27: 81–96.
- Oksanen, J., F. G. Blanchet, M. Friendly, R. Kindt, P. Legendre, D. McGinn, P. R. Minchin, R. B. O'Hara, G. L. Simpson, P. Solymos, M. H. H. Stevens, E. Szoecs & H. H. Wagner, 2017. *Vegan: Community ecology Package*. R package version 2.4-3. Available at <http://CRAN.R-project.org/package=vegan>.
- Piet, G. J., 1998. Ecomorphology of a size-structured tropical freshwater fish community. *Environmental Biology of Fishes* 51: 67–86.
- Post, D. M., C. A. Layman, D. A. Arrington, G. Takimoto, J. Quattrochi & C. G. Montaña, 2007. Getting to the fat of the matter: models, methods and assumptions for dealing with lipids in stable isotope analyses. *Oecologia* 152: 179–189.
- R Development Core Team, 2016. *R: A language and environment for statistical computing*. Available at <https://r-project.org>.
- Ribbink, A. J., 1990. Alternative life-history styles of some African cichlid fishes. *Environmental Biology of Fishes* 28: 87–100.
- Seegers, L., L. De Vos & D. O. Okeyo, 2003. Annotated checklist of the freshwater fishes of Kenya (excluding the lacustrine haplochromines from Lake Victoria). *Journal of East African Natural History* 92: 11–47.
- Spataru, P., 1976. The feeding habits of *Tilapia galilaea* (Artedi) in Lake Kinneret (Israel). *Aquaculture* 9: 47–59.
- Spataru, P. & M. Zorn, 1978. Food and feeding habits of *Tilapia aurea* (Steindachner) (Cichlidae) in Lake Kinneret (Israel). *Aquaculture* 13: 67–79.
- Strayer, D. L., 2010. Alien species in fresh waters: ecological effects, interactions with other stressors, and prospects for the future. *Freshwater Biology* 55: 152–174.
- Trewavas, E., 1983. *Tilapiine Fishes of the Genera Sarotherodon, Oreochromis and Danakilia*. British Museum of Natural History, London.
- Utermöhl, H., 1931. Neue Wege in der quantitativen Erfassung des Plankton (mit besonderer berücksichtigung des Ultra-planktons). *Verhandlungen der Internationale Vereinigung für Theoretische und Angewandte Limnologie* 5: 567–596.
- Utermöhl, H., 1958. Zur Vervollkommnung der quantitativen Phytoplankton-Methodik. *Mitteilungen der Internationale Vereinigung für Theoretische und Angewandte Limnologie* 9: 1–38.
- van Bree, L., F. Peterse, M. T. J. Van der Meer, J. J. Middelburg, A. M. D. Negash, W. De Crop, C. Cocquyt, J. J. Wieringa, D. Verschuren & J. S. Sinningh-Damsté, 2018. Seasonal variability in the abundance and stable carbon-isotopic composition of lipid biomarkers in suspended particulate matter from a stratified equatorial lake (Lake Chala, Kenya/Tanzania): implications for the sedimentary record. *Quaternary Science Reviews* 192: 208–224.
- Venables, W. N. & B. D. Ripley, 2002. *MASS: Modern Applied Statistics with S*. 4th edition. Available at <https://www.stats.ox.ac.uk/pub/MASS4>.
- Vitousek, P. M., H. A. Mooney, J. Lubchenco & J. M. Melillo, 1997. Human domination of Earth's ecosystems. *Science* 277: 494–499.
- Witte, F., T. Goldschmidt, P. C. Goudswaard, W. Ligtoet, M. J. P. Van Oijen & J. H. Wanink, 1991. Species extinction and concomitant ecological changes in Lake Victoria. *Netherlands Journal of Zoology* 42: 214–232.
- Wolff, C., G. H. Haug, A. Timmermann, J. S. Sinningh-Damsté, A. Brauer, D. M. Sigman, M. A. Cane & D. Verschuren, 2011. Reduced interannual rainfall variability in East Africa during the Last Ice Age. *Science* 333: 743–747.
- Wolff, C., I. Kristen-Jenny, G. Schletter, B. Plessen, H. Meyer, P. Dulski, R. Naumann, A. Brauer, D. Verschuren & G. H. Haug, 2014. Modern seasonality in Lake Challa (Kenya/Tanzania) and its sedimentary documentation in recent lake sediments. *Limnology and Oceanography* 59: 1621–1636.
- Zengeya, T. A., A. J. Booth, A. D. S. Bastos & C. T. Chimimba, 2011. Trophic interrelationships between the exotic Nile tilapia, *Oreochromis niloticus*, and indigenous tilapiine cichlids in a subtropical African river system (Limpopo River, South Africa). *Environmental Biology of Fishes* 92: 479–489.